Guidelines for Protocols Involving Antimalarial Drug Level Determination

Sample collection techniques

The assays will enable the investigators to correlate blood levels of anti-malaria drugs with the clinical outcome of treatment. Collection of samples for the assays should be done in a separate location from patient treatment to minimise contamination of sample during drug administration.

Sample Collection and Data points:

The blood-sampling schedule will be specific to each drug. Determination of anti-malaria concentrations will be by standard HPLC analysis with UV detection. PK parameter analysis will be performed using appropriate software (eg Topfit®).

Samples will be obtained from patients before drug administration and during follow-up period (days 0, 1, 3, 7, 14, 21 and 28) or any other time when there is recrudescence of parasitemia). For example:

- *Chloroquine, amodiaquine:* days 0, 1 (prior to dosing*), and 3, 7, 14, 21 and 28
- *Sulfadoxine/pyrimethamine:* 0 hour (prior to dosing*), and 1 3, 7, 14, 21 and 28

  **Note: It's recommended that blood samples be collected from patients before drug administration.*

A sample volume of 100ul will be spotted for all drugs except SP which, will be collected by venupuncture. Blood samples collected from finger prick will be spotted with a micropipette in triplicate unto filter paper and stored until analysed. Samples should be analysed within 3 months of collection especially for SP.

Sample handling:

The samples will be labelled clearly for identification with:

a) Patient ID or study reference number.
b) Study drug.
c) Date and sample time after dose.
d) Volume of sample blotted (filter paper-absorbed samples).

Labels must be clear and legible to constitute an 'acceptable sample'. After labelling, samples will be stored d esiccated at room temperature protected from dust, moisture and direct light.

Protocols for Analysis

1. **Chloroquine (CQ) Assay** (100 ul whole blood absorbed on filter paper)**.**

Sample preparation, Extraction and Chromatographic conditions. – Filter paper absorbed samples:

- blot the internal standard on the filter paper sample, quinidine (QD) is appropriate.
- allow to dry and cut up the blotted sample into small pieces,
- transfer into a silinized glass tube and soak in 1ml 0.2M HCl for 10-15 min.
- add 100µl 6M NaOH and vortex mix for 30sec.
- extract with 5ml n-Hexane: tert butylether (1:1 v/v) by vortexing for 1min or inversion mixing for 10-15min.
- transfer the organic layer into fleshly silinized glass tube and evaporate to dryness under N_2.
- reconstitute the residue with 50-100 µl of mobile phase and inject appropriate volume onto the column.

Conditions:

Column: Ultrashere ODS 5µ 15cm x 4.6mm.

Mobile phase: 90% H_2O : 10% Acetonitrile + 1% triethylamine, adjust pH to 2.8 with orthophosphoric acid.

Flow rate: 2ml/min.

Detection: UV @ 340nm

Retention Times: Peak A, CQ @ 3mins after injection event.
Peak B, QD @ 4mins after injection event.

2. **Pyrimethamine Assay** (200 ul of plasma)**.**

Sample preparation, Extraction and Chromatographic conditions.

- add internal standard (proguanil),
- transfer into a silinized glass tube and soak in 1ml of ammonia for 10– 15 min.
- mix and extract with 5 ml ethyl acetate.
- proceed as for CQ assay above.

Conditions:

Column: Ultrashere ODS 5µ 15cm x 4.6mm.

Mobile phase: 50% H_2O : 40% Acetonitrile: 10% Methanol + 1g/l 1-Octensulphonic acid, adjust pH to 3.8 with HCl.

Flow rate: 1ml/min.

Detection: UV @ 210nm

Retention Times: Peak A, pyrimethamine @ 4.5mins after injection event.
Peak B, proguanil @ 6.5mins after injection event.

3. Sulfadoxine Assay (50 ?l of plasma)**.**

Sample preparation, Extraction and Chromatographic conditions.

- ? add internal standard (sulfisoxazole).
- ? add 0.1M sodium acetate buffer (pH 3.6-5.6) for 10-15 min.
- ? extract with 5 ml n-hexane:ethyl acetate (1:1v/v).
- ? proceed as for CQ assay above

Conditions:

Column: Ultrashere ODS 5µ 15 cm x 4.6 mm.

Mobile phase: composition: 70% H_2O : 19% Methanol : 11% Acetonitrile: + 1g/l 1-Octensulphonic acid, adjust pH to 3.0 with HCl.

Flow rate: 1.5ml/min.

Detection: UV @ 254 nm

Retention Times: Peak A, sulfisoxazole @ 1.5 mins after injection event.
Peak B, sulfadoxine @ 2.5 mins after injection event.

Sample analysis

? Determination of antimalarial drugs levels in biological fluids requires established laboratory for specific, sensitive and reliable analytical methods that are suitable for the quantitative detection of drugs and their (active) metabolites. ***In places where there are no such facilities for drug analysis, samples should be transported to designated laboratory for analysis.*** The Malaria Research laboratories, College of Medicine, University of Ibadan, Nigeria, will serve as the network centre for the analysis and training of researchers for pharmacokinetic analysis.

Data analysis

The primary objective of the determination of antimalarial drug concentration (in whole blood) is to define the pharmacokinetic / pharmacodynamic correlation, i.e., correlation between drug concentration and clinical response. The collected data points from large number of patients would also allow the investigation of the disposition of the trial drug and the influence of disease (malaria) by applying "Population Pharmacokinetic" approach.

Quality Assurance

? *Methodology of drug analysis:* Internationally approved quality control procedures/ assays should be in place and described with a detailed written "Standard Operating Procedure" (SOP) and should meet predetermined levels of acceptable variability. Sample analysis should be done with incorporated regular quality control, with samples sent to a quality control laboratory and blinded samples sent to sites. The analytical centre will be audited and monitored for quality control specifically for the assays used in the study.

Requirements for determination of drug levels

? High Performance Liquid Chromatography unit with ultraviolet and /or fluorescence detection.

? Uninterrupted power supply,

? Screw-capped glass tubes for sample extraction

? Centrifuge

? Vortex mixture

? Sample evaporator and nitrogen supply

? Mechanical tumbler

? Freezer (-20 ^{o}C)

? Fume hood

? HPLC grade reagents (acetonitrite, triethylamine, orthophosphoric acid etc)

? Drug standards (Quinidine, sulfadoxine, pyrimethamine, proguanil, salfazoxazole, chloroquine and desethyl-chloroquine)

Table 2 Determination of antimalarial drug concentrations

Drug	Sample Matrix	Sample volume (ul)	No. of samples per patient	No. of analysis	Methods	Sampling times (hours)	Storage of Samples (^{O}C)
Chloroquine	Whole blood on filter paper	100	7 (plus additional sample on the day of recurrence,if occur)	2 (chloroquine, n-desethylchloroquine)	HPLC-UV	0, 24, 72, 168, 336, 504 and 672	-20 to –40
Sulfadoxine/ Pyrimethamine	Plasma sample from venous bloodWhole blood on filter paper	50 for sulphadoxine & 200 for pyrimethamine	7 (plus additional sample on the day of recurrence,if occur)	2 (sulfadoxine, pyrimethamine)	HPLC-UV	0, 24, 72, 168, 336, 504 and 672	-20 to –40
Amodiaquine	Whole blood on filter paper	100	7 (plus additional sample on the day of recurrence,if occur)	2 (amodiaquine, n-desethylamodiaquine)	HPLC-UV	0, 24, 72, 168, 336, 504 and 672	-20 to -40